ANNEX D

JOINT MEDICAL CHEMICAL, BIOLOGICAL, AND NUCLEAR DEFENSE RESEARCH PROGRAMS

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JOINT MEDICAL CHEMICAL, BIOLOGICAL, AND NUCLEAR DEFENSE RESEARCH PROGRAMS

The joint medical chemical, biological, and nuclear (radiological) defense research programs are each addressed in the next three sections.

D.1 MEDICAL CHEMICAL DEFENSE RESEARCH PROGRAM

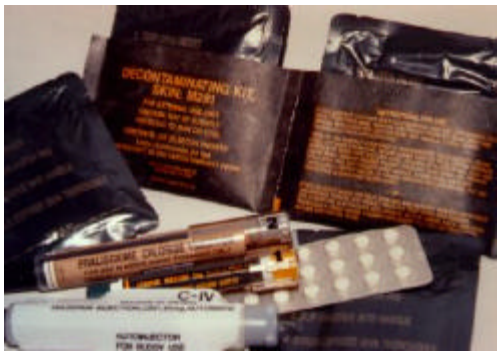
D.1.1 Fielded Products

Advances in medical research and development (R&D) significantly improve the warfighting mission by sustaining unit effectiveness through conserving the fighting strength of our forces and supporting the nation's global military strategy, which requires the ability to effectively deploy and operate. Medical R&D products (materiel and non-materiel solutions) provide the foundation that ensures the fielding of a flexible, sustainable, modernized force across the spectrum of conflict and in the full breadth and depth of the battlefield. Overcoming medical threats and extending human performance has provided a significant increase in military effectiveness in the past and presents the potential for future enhancement of military operational effectiveness. Some fielded materiel and non-materiel solutions by medical chemical defense R&D are:

Pharmaceuticals (See Figure D-1):

- Nerve Agent Antidote Kit (Mark I), 1983
- Skin Decontamination Kit (M291), 1990
- Nerve Agent Pretreatment (Pyridostigmine), (NAPP), 1985*
- Convulsant Antidote for Nerve Agent (CANA), 1991*
- Medical Aerosolized Nerve Agent Antidote (MANAA), 1994*
- Test Mate® ChE (Cholinesterase) Kit, 1997

* Initial fielding of these medical products was funded under Low Rate Initial Production (LRIP) options in developmental contracts with RDT&E dollars. Therefore, the following FY96 actions were accomplished: (1) Proved long term extended stability of the medical aerosolized nerve agent antidote (MANAA), the convulsant antidote for nerve agent (CANA), and the nerve agent pretreatment (pyridostigmine), (2) Completed one year follow-up to pyridostigmine gender study, and (3) Submitted new drug application (NDA) for pyridostigmine to the FDA.



MARK I, M291, NAPP, and CANA

Test Mate® ChE Kit

Figure D-1. Selected Fielded Pharmaceutical Products

Materiel (See Figure D-2):

- Resuscitation Device, Individual, Chemical, 1990
- Decontaminable Patient Litter (NSN 6530-01-380-7309), 1991
- Chemical Warfare (CW) Protective Patient Wrap (NSN 8415-01-311-7711), 1991
- Computer-Based Performance Assessment Battery, 1993
- M40 Protective Mask Vision Correction (optical inserts)

Figure D-2. Decontaminable Patient Litter and CW Patient Wrap

D.1.2 Medical Chemical Defense Research and Development Accomplishments

The medical chemical defense research and development technical barriers and accomplishments during FY97 are grouped by the classical chemical threat categories, which include the following:

- Vesicants or blister agents (*e.g.*, sulfur mustard [HD] and Lewisite),
- Nerve agents (*e.g.*, soman [GD], VX),
- Blood agents (*e.g.*, cyanide), and
- Respiratory agents (*e.g.*, phosgene).

The chemical threat, however, is not restricted to commonly accepted classical agents. Novel agents may be developed by potential adversaries. The ability to provide timely and effective medical countermeasures to new threats depends upon maintaining a high level of technological capability.

Countermeasures to these threats include pharmaceuticals, medical equipment, specialized materiel or medical procedures, and concepts for training, doctrine, and organization. Medical countermeasures are designed not only to prevent lethality but to preserve and sustain combat effectiveness in the face of combined threats from chemical and conventional munitions on the integrated battlefield by:

- Prevention of the effects of chemical agents (*e.g.*, pretreatments or prophylaxes),
- Far-forward treatment upon exposure to chemical warfare threats (*e.g.*, antidotes),
- Chemical casualty care (*e.g.*, therapy and management).

THREAT CATEGORY: VESICANT AGENTS

The countermeasures, technical barriers, and accomplishments in the chemical threat category of vesicant agents are outlined below.

Countermeasures:

- Reactive topical skin protectant for blister agents.
- Products that prevent or moderate vesicant injury.

Technical Barriers:

- Appropriate model systems for testing treatment efficacy and safety in humans.
- Quick-acting and long-lasting antidotes that are easy to carry and use on the battlefield.

Accomplishments:

- Using artificial skin models and simulant to sulfur mustard (HD), determined that regulatory processes for cytokine antagonists are as critical as those for primary cytokines in biological responses to vesicant injury.
- Developed human whole blood model for assessing vesicant injury-induced changes in cytokine responses.
- Demonstrated that 7 of 33 compounds tested in the mouse ear screen provided significant reductions in HD-induced edema, histopathology, or both.
- Demonstrated that N-acetyl cysteine provided protection against HD vapor inhalation when assessed through biochemical changes in the rat bronchoalveolar lavage model.
- Showed that cytoprotection of keratinocytes against HD by the calcium chelator BAPTA appears to be related to inhibition of cellular metabolic processes and cell division.
- Demonstrated that pulsed CO_2, laser debridement of weanling pigskin exposed to HD vapor significantly improved viability and organization of the healing epidermis.

- Demonstrated elevations of inflammatory cytokine leukotriene B4; and the chemotactic complement component C5a in normal human fibroblasts exposed *in vitro* to HD, which could account for the inflammatory response to cutaneous HD exposures.
- Demonstrated elevation of Fc receptors and receptors for the complement component C1q in keratinocytes exposed *in vitro* to HD.
- Developed a single-cell comet electrophoresis assay that allowed time and concentration demonstrations of DNA strand breaks following *in vitro* exposure of lymphocytes to HD.
- Measured HD-specific changes in gene expression using the differential display polymerase chain reaction (DDPCR), which may allow detection of alteration in human keratinocytes exposed *in vitro* to HD.
- Found unexpectedly high levels (10-fold elevation) of the DNA repair enzyme methyl guanine methyltransferase (MGMT) in human keratinocytes as compared to levels seen in other human tissues.
- Developed a pulse field gel electrophoresis assay for measurement of DNA double-strand breaks following HD-exposure that revealed a necrotic pattern of DNA damage at 24 hours, suggesting that double-strand breaks are only evident at earlier time points.
- Found that exposure of cultured keratinocytes to HD failed to generate a change in intracellular calcium, but may result in transmembrane changes of the chelating indicator dyes.
- Determined that HD exposed keratinocytes produced an 80-Kd calcium-dependent serine protease that has been chromatographically purified and sequenced.
- Demonstrated that expression of keratin 14 is decreased within 1 hour of HD exposure in cultured keratinocytes.
- Developed methods for analysis of multiple biochemical markers, including serum amyloid A and myeloperoxidase, from a single punch biopsy following cutaneous exposure to HD.
- Showed that pretreatment of lymphocytes with L-oxothiozolidine-4-carboxylate, a cysteine precursor, provides a level of protection against HD cytotoxicity.
- Showed that anionic sulfur compounds interact directly with DNA, possibly changing the molecular topology, which could be the basis for their efficacy in protecting against cellular damage by HD.
- Identified three tissue fixatives, which allow qualitative measurement of the extent of injury following sulfur mustard (HD) exposure in the porcine model.
- Developed a procedure for measuring cessation of offgassing of HD following animal exposure experiments.
- Developed gas chromatographic-mass spectrometric (GC-MS) method to document lewisite exposure levels by detection, extraction and derivatization of 2-chlorovinylarsonous acid (CVAA) in the urine of guinea pigs exposed to lewisite.
- Installed atomic absorption (AA) instrumentation to measure total arsenic content of biological samples following lewisite exposure to complement the GC-MS procedure.
- Evaluated clinical endpoints utilized in human intensive care in the miniature swine inhalation model.
- Showed that exposure of keratinocytes to HD leads to cytotoxicity involving terminal differentiation and apoptosis via a calcium-calmodulin and caspase-dependent pathway

(Dr. M.E. Smulson, Georgetown University).

- Assessed the toxicokinetics of HD in the hairless guinea pig following I.V. administration of 0.3 LD_{50} using gas chromatography coupled with pulsed flame photometric detection (PFPD); showed that half-lives of distribution and elimination were 0.7 and 152 minutes, respectively (Dr. J. Langenberg, TNO, The Netherlands).
- Observed dramatic increases in levels of the cytokines IL-1b, IL-6, TNF-alpha, and MIP-1a mRNA following cutaneous HD exposure in the mouse ear (Short Term Analytical Service [STAS], Casillas/Sabourin, Ohio State University).
- Found elevation of two precursor enzymes for substance P following cutaneous HD exposure in the mouse ear (STAS, Casillas/Cutler and Pollack, Mercer University, GA).

THREAT CATEGORY: NERVE AGENTS

The countermeasures, technical barriers, and accomplishments in the chemical threat category of nerve agents are outlined below.

Countermeasures:

- Pretreatment regimen that protects against incapacitating effect.
- Medical countermeasures to minimize lethality, morbidity, and residual incapacitation.

Technical Barriers:

- Appropriate experimental model systems to predict pretreatment, drug or treatment efficacy and safety in humans.

Accomplishments:

- Accomplished Milestone Zero transition of potent centrally acting anticholinergic drugs as treatment for nerve agent-induced seizures.
- Identified three clinically used anticholinergic compounds for testing as advanced anticonvulsant in nonhuman primates.
- Performed pharmacokinetic/pharmacodynamic analysis of anticonvulsant drugs to determine blood levels necessary for clinical efficacy against nerve agent seizures.
- Determined that the mechanisms for seizure initiation and development of brain damage are essentially identical for all nerve agents.
- Evaluated utility of use of the enzyme troponin as a clinical marker for nerve agent-induced cardiac damage.
- Initiated preparation of new mutants of human butyrylcholinesterase to enhance their catalytic properties against nerve agents.
- Continued collaboration on mutants of human butyrylcholinesterase and acetylcholinesterase (USAMRICD, University Nebraska, Israeli Institute of Biological Research, and Centre Recherches du Service de Sante des Armees, France).
- Collaborated with the University of Michigan on the characterization of a paraoxonase from dog liver.

- Developed a physiologically based pharmacokinetic model for the stereoisomers of soman.
- Determined the concentration of the nerve agent bioscavenger, carboxylesterase, in liver and plasma of seven mammalian species. The pattern of the liver/plasma ratio of carboxylesterase suggested that plasma levels of carboxylesterase are regulated by liver secretion.
- Synthesized the peptide His-Ile-Glu-Leu and found it could induce complete displacement of carboxylesterase from liver microsomes at a concentration of 5×10^{-6} M.
- Determined by ^{14}C-soman binding to liver microsomes that the threefold lower level of soman binding in liver of rats vs. guinea pigs and monkeys may explain the threefold slower terminal elimination rate of soman in rats vs. these other species.
- Initiated development of immunoaffinity purification for carboxylesterase (CaE).
- Tested two more chiral capillary GC columns coated with 3-beta cyclodextrin derivatives for stereoisomer separation of soman, sarin, tabun and GF. The butyryl 3 gamma cyclodextrin remains the best column for separating soman stereoisomers.
- Characterized a monoclonal anti-soman antibody in an enzyme linked immunosorbant assay (ELISA) assay for binding sublethal concentrations of soman and optimized experimental parameters.
- Collaborated on a study of paraoxonase polymorphism in a population of farm workers in the state of Washington. Found two isozymes, one active against paraoxon, and one active against nerve agent.
- Produced a double mutant of CaE having a histidine near the active site and an altered C-terminal residue that retains CaE activity and is secreted in a transient expression system.
- Completed development of software for physiological-based pharmacokinetic model for all four soman stereoisomers and initiated determinations of stereospecific biochemical parameters for soman.
- Demonstrated that administration of equine butyrylcholinesterase at levels known to protect against 5 LD_{50}s of soman had no behavioral side effects in a nonhuman primate model.
- Optimized fetal bovine acetylcholinesterase (FB-AChE) and equine butyrylcholinesterase catalytic hydrolysis of organophosphorus compounds in the presence of an appropriate oxime by attachment to a solid support.
- Determined the subcutaneous median lethal doses of four classified novel agents in guinea pigs and rats.
- Identified the biochemical mechanism of action of the novel agents as inhibitors of acetylcholinesterase.
- Performed kinetic measurements of the inhibition of acetylcholinesterase by novel agents to rank-order these agents against standard chemical warfare agents.
- Determined the ability of current medical countermeasures (*i.e.*, atropine, oximes, pyridostigmine) to protect against the lethal effects of novel agents in guinea pigs.
- Published a report characterizing the toxicity and medical treatment of a novel agent that is a structural isomer of VX in the *Journal of American College of Toxicology*.
- Developed GC/MS method for detection and quantitation of diisopropylaminoethyliol (DPAT), a metabolite of VX in plasma.

- Determined tissue/blood partition coefficients for soman in liver, kidney, lung, brain and muscle of rodents and nonhuman primates to aid in extrapolation of soman pharmacokinetics to humans (Dr. L. De Jong, TNO, The Netherlands).
- Implemented a Short Term Analytical Services (STAS) contract with The Catholic University of America for "Determination of the Origins of the Inhibition of Cholinesterase by Organophosphates" (Dr. Kovach).
- Determined that cholinesterase is involved in neuronal development, even when it may lack catalytic activity (Dr. H. Soreq, Hebrew University, Israel).
- Elucidated the role of Aspartate 70 in the binding and hydrolysis of succinyldithiocholine by butyrylcholinesterase (BuChE), explaining why patients with an atypical variant of human BuChE respond abnormally to succinyldicholine (Dr. O. Lockridge, University of Nebraska).
- Determined that the normal tetramerization of BuChE occurs at the C-terminus, that tetramerization is not essential for activity, and that the N-terminus cannot be altered without serious repercussions with respect to activity (Dr. O. Lockridge, University of Nebraska).
- Prepared and delivered to the Weizmann Institute for crystallographic studies more than 100 mg of pure human acetylcholinesterase (h-AChE). These crystals are diffractible at about 2.7 A (Dr. A. Shafferman, IIBR, Israel).
- Cloned 2,6 sialyltransferase into human kidney-293 cells that express h-AChE so that the enzyme is fully capped at the glycosylation site. The capped enzyme has a significantly improved biological half-life in mice (Dr. A. Shafferman, IIBR, Israel).
- Determined that when soman-inhibited AChE undergoes aging, the pinacolyl group is lost and is not bound at some other site within the enzyme (Dr. A. Shafferman, IIBR, Israel).
- Crystallized mouse AChE without the use of fasciculin, a snake toxin peripheral site inhibitor. This is the first mammalian AChE to be crystallized without fasciculin and these crystals are now being used for crystallography studies in Grenoble (Dr. P**.** Taylor, University of California at San Diego, UCSD).
- Determined that differences in rates of reactivation of chiral inhibitors of AChE by oximes are due to the angle of attack on the phosphonate bond, which is dictated by side chains in the enzyme (Dr. P. Taylor, UCSD).
- Demonstrated that nerve agent-induced seizures alter the clearance of metabolic anions from the brain, which increases the oxidative stress on neural tissue and contributes to brain damage (Dr. T. Pazdernik, University of Kansas Education Center).
- Discovered a population of n-methyl-d-aspartate (NMDA) binding sites that are sensitive to homoquinolate and are insensitive to displacement by glutamate. These sites are selective to anatomical regions that suffer severe neural damage following nerve agent seizures (Dr. D. Monaghan, University of Nebraska Education Center).
- Developed molecular probes to anatomically localize different subtypes of NMDA receptors for use in labeling and anatomical localization of different receptor subtypes in areas of the brain involved in nerve agent seizures (Dr. D. Monaghan, University of Nebraska Education Center).

THREAT CATEGORY: BLOOD AGENTS

The countermeasures, technical barriers, and accomplishments in the chemical threat category of blood agents are outlined below.

Countermeasures:

- Pretreatment compounds to protect against rapid action of these chemical agents

Technical Barriers:

- Appropriate experimental model systems to predict drug or treatment efficacy and safety in humans
- Pretreatments/antidotes with special characteristics, such as quick action, long-lasting, easy to carry and use

Accomplishments:

- Developed a prototype, noninvasive finger-cuff optical probe, under contract with Omeda Corp., to simultaneously monitor continuous measurements of oxyhemoglobin, deoxyhemoglobin, methemoglobin and carboxyhemoglobin for use in cyanide exposure.

THREAT CATEGORY: RESPIRATORY AGENTS

The countermeasures, technical barriers, and accomplishments in the chemical threat category of respiratory agents are outlined below.

Countermeasures:

- Short-term: Health risk criteria for emerging threat doctrine, care and treatment strategies
- Intermediate-term: Specific casualty management techniques to improve survival and minimize lost duty time
- Long-term: Pharmaceutical/biological pretreatments, antidotes, or decontaminants/ protectants

Technical Barriers:

- Appropriate experimental model systems to predict drug or treatment efficacy and safety in humans
- Pretreatment/antidotes with special characteristics, such as quick action, long-lasting, and easy to carry

Accomplishments:

- Demonstrated significant reduction in pulmonary edema formation following treatment of mice with ibuprofen after exposure to phosgene.
- Prevented oxidation of glutathione in ibuprofen-treated phosgene-exposed mice.
- Reduced phosgene-induced pulmonary edema in mice by dietary pretreatment with butylated hydroxyanisole or n-propyl gallate.

D.1.3 Advanced Development Products

In advanced development, the goal is proof-of-principle and conducting all studies necessary to obtain FDA approval/licensure of drugs and vaccines. The medical R&D process links the materiel developer (U.S. Army Medical Research and Materiel Command (USAMRMC)) with the combat and training developer (Army Medical Department Center and School (AMEDDC&S)) and the logistician in addressing the threat and Department of Defense (DoD) requirements. Medical chemical defense products now in the advanced development phase are the following:

PRODUCT: TOPICAL SKIN PROTECTANT (TSP)

Concept:

- Use perfluorinated formulations.
- Form non-toxic, non-irritating barrier film layer on skin.
- Augments Mission Oriented Protective Posture (MOPP).
- Protection against vesicant and nerve agents.

Status:

- Two candidates transitioned to demonstration-validation phase.
- Candidates demonstrated efficacy against broad spectrum of threat agents; down-selected to one candidate.
- Investigational New Drug (IND) application submitted to the FDA.
- Demonstrated the human safety and technical performance of the topical skin protectant.
- Demonstrated extended stability of the topical skin protectant.
- Validated production/manufacturing capability for the topical skin protectant.
- Awarded a manufacturing development contract.
- New Drug Application is under preparation.

PRODUCT: MULTICHAMBERED AUTOINJECTOR

Concept:

- Speed administration of life-saving antidotes against nerve agents.
- Replace two Injector Mark I Nerve Agent Antidote Kit with single autoinjector.

Status:

- Engineering contract awarded in September 1993.
- Fielding will require full FDA approval.
- Demonstrated the human safety of the multi-chambered autoinjector.
- Engineering and development of final prototype completed.

PRODUCT: CYANIDE PRETREATMENT

Concept:

- Provide protection against incapacitation and lethality without performance degradation.
- Enhance soldier protection and sustainment.

Status:

- Completed pre-clinical toxicology and drug distribution studies.
- Developed dose parameters and performance assessments.
- Concluded animal toxicology studies for cyanide pretreatment.
- Completed preparation of Investigational New Drug Application.
- Initial efforts to conduct first human safety tests.
- Draft Engineering and Manufacturing Development Request For Proposals undergoing staffing.

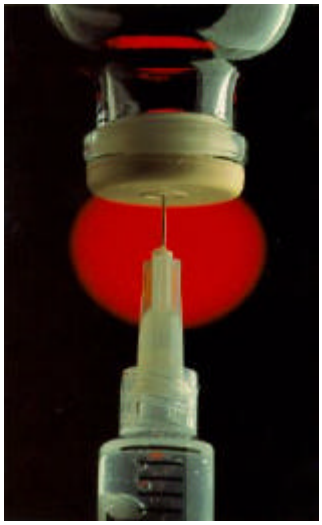
D.2 MEDICAL BIOLOGICAL DEFENSE RESEARCH PROGRAM

D.2.1 Biological Defense Products

Advances in DoD medical R&D significantly impact the warfighting mission by sustaining unit effectiveness through conserving the fighting strength of our soldiers and supporting the nation's global military strategy which requires the ability to effectively deploy and operate. Medical R&D products (materiel and non-materiel solutions) provide the foundation that ensures the fielding of a flexible, sustainable, modernized force across the spectrum of conflict and in the full breadth and depth of the battlefield. Overcoming medical threats and extending human performance has provided a significant increase in military effectiveness in the past, and presents the potential for future enhancement on military operational effectiveness. Some of the solutions developed by medical biological defense R&D include the following:

Vaccines:

- Anthrax Vaccine (licensed)
- Botulinum Toxoid Vaccine, Pentavalent (IND #3723)
- Botulinum Type F Toxoid Vaccine (IND #5077)
- Botulism Antitoxin, Heptavalent Equine (Types A, B, C, D, E, F, and G) (IND #3703)
- Botulism Immune Globulin, Human (IND #1332)
- Eastern Equine Encephalitis Virus Vaccine (IND #266)
- Q Fever Vaccine, Purified Whole Cell, CM Residue, Formalin Inactivated, Gamma Irradiated (IND #3516)
- Tularemia Vaccine (IND #157)
- Vaccinia Virus Vaccine, Cell Cultured (IND #4984)
- Venezuelan Equine Encephalomyelitis Virus Vaccine, TC-83 (IND #142)
- Western Equine Encephalitis Virus Vaccine (IND #2013)



D.2.2 Biological Defense Research and Development Accomplishments

The biological defense research and development technical barriers and accomplishments during FY97 are grouped by biological threat category, which include the following:

- Bacterial (and rickettsial) agents,
- Protein toxins, and
- Viral agents.

In addition, research and development accomplishments in the area of confirmatory assays for biological warfare threat agents is presented at the end. The objective of this effort is to develop the capability to confirm in biological samples the initial field diagnosis of a biological warfare threat agent.

DARPA is pursuing multi-agent and broad-spectrum approaches, both to defend against current known threats and to anticipate potential future threats. Accomplishments of DARPA BWD programs for FY97 include the following:

Medical Countermeasures:

Demonstrated the feasibility of modified red blood cells to eliminate a model pathogen (bacteriophage) from the circulation. In an animal model, more than 99.9% clearance of circulating virus was achieved in less than 1 hour.

Demonstrated feasibility of transfecting stem cells *in vitro* to express new gene products, in order to develop modified stem cells to produce therapeutic products or provide automatic "booster" immunizations.

Consequence Management Tools:

ENCOMPASS (Enhanced Consequence Management Planning and Support System), an integrated set of consequence management tools, was developed and demonstrated with CBIRF (Marine Corps Chemical and Biological Incident Response Force). ENCOMPASS was used in Denver by CBIRF during the Summit of the Eight (June 1997) to provide plans, situational awareness and patient management in the event of a chemical or biological incident.

THREAT CATEGORY: BACTERIAL AGENTS

The countermeasures, technical barriers, and accomplishments in the biological threat category of bacterial agents are outlined below.
Countermeasures:

- Vaccines for immunity against threat agents
- Antibiotics for treatment of bacterial diseases
- Forward deployed diagnostic systems

Technical Barriers:

- Incomplete genetic information for all the threat agents
- Appropriate animal model systems for investigation of some bacterial threats and countermeasures
- Capability to produce Good Manufacturing Practice (GMP) pilot lots of vaccine candidates
- Inability to perform human clinical trials to prove efficacy of vaccines

- Difficulty in optimizing and comparing different expression vectors for recombinant products
- Difficulty in field testing rapid identification kits under natural conditions
- Defining surrogate markers of protection

Anthrax

Accomplishments:

- Prepared synthetic peptides and corresponding monoclonal antibodies for the antigenic region of protective antigen (PA), and used the antibodies to demonstrate that the neutralizing epitope on the PA protein is conformational, and located in the region of amino acid residues 168-237.
- Initiated study of the potency and stability of recombinant PA plus Alhydrogel over a 1 year time period, and found that inclusion of formaldehyde at permissible levels enhanced immunogenicity. Established the rabbit model for passive immunity studies in order to assess surrogate markers of immunity.
- Determined that immune serum derived from humans immunized with the licensed anthrax vaccine did not inhibit spore germination any more than did sera from non-immune individuals, suggesting that the vaccine does not elicit antibodies to spore-specific antigens.
- Successfully cloned and demonstrated the activity of a construct of the anthrax plasmid pXO2 origin of replication, which will facilitate construction of a shuttle vector for further study of genes for antigens in *Bacillus anthracis* vaccine strains. Demonstrated proof of principle by successfully cloning the non-toxic, immunogenic heavy chain C-terminal of botulinum toxin into a vaccine strain of *B. anthracis*.

Plague

Accomplishments:

- Determined that there are three distinct genetic classes of the F1-negative strains of *Yersinia pestis*, and identified and characterized the genetic basis for the different types of V antigen associated with different strains of *Yersinia* species and different strains of *Y. pestis*.
- Tested the ability of purified F1 protein, which bears biochemical similarities to the IL-1 receptor antagonist called IL-1ra, to activate mononuclear cells or regulate the inflammatory response, and found that F1 did not stimulate cells or affect cytokine production.
- Investigated the physiological basis of expression of the virulence factors Yop proteins and V antigen, and linked the expression of these factors to the organism's calcium and temperature sensitivities.
- Initiated efforts to refine the candidate V and F1-V fusion protein antigens, ensuring that the protein sequence of the V antigen is 100% correct and that the purification procedure eliminates unwanted components.

- Evaluated the immune response of mice which survived lethal *Y. pestis* challenge after antibiotic treatment, and found that presence or levels of antibodies to 6 of the 13 antigens screened produced positive results. This information may prove useful in selecting antigens for vaccine candidates and for development of improved serologic diagnostic assays.
- Developed *in vitro* and *in vivo* models with which to characterize the host immunosuppressive activity of the V antigen, a major virulence factor, and demonstrated a direct inhibitory effect of V protein on host cell chemotaxis.
- Identified six different plague virulence factors as suitable candidates for further cloning into a novel yeast expression system which will allow more refined study of the mechanism of action of these virulence factors.
- Established experimental approaches to characterization of the molecular mechanics of secretion of *Y. pestis* virulence factors, which, when fully defined, will facilitate development of candidate live, attenuated vaccine strains of plague.
- Demonstrated protection of mice from lethal aerosol challenge with *Y. pestis* for at least a year by immunizing them with a single dose of either recombinant F1 plus V antigens or the recombinant F1-V fusion protein.
- Determined that the beta-lactam antibiotic, ceftriaxone, accelerated mortality in mice with pneumonic plague when treatment was initiated late in the course of the disease, and showed that all beta-lactam antibiotics tested produced similar effects.

Glanders

Accomplishments:

- Determined the *in vitro* antibiotic sensitivities for *Burkholderia mallei* strains China 7 and Budapest, and identified tobramycin sulfate, doxycycline hycolate, ofloxacin, ciprofloxicin and amikacin sulfate to be effective.
- Initiated development of an ELISA for serodiagnosis of glanders and determined that the assay appears to be sensitive for identification of antibodies against *B. mallei*; specificity and other characteristics of the test remain to be determined.
- Characterized the mouse and hamster animal models of glanders infection, finding that less than 10 colony-forming units were required for lethal infection by injection of hamsters, whereas approximately a one hundred thousand-fold higher dose was required for lethal infection by injection of most mouse strains. One mouse strain appeared to be resistant to challenge; the Burkholderia-sensitive BALB/c mouse strain was chosen for further evaluation.
- Characterized the pathologic changes in hamsters after *B. mallei* infection, and noted that virtually all tissues were ultimately affected at later time points, particularly vascular tissues.
- Demonstrated that bacteria could be isolated from the liver within hours of experimental infection of animals, and that spleen, lung and blood were bacteremic over the course of the subsequent 24-48 hours.

Brucellosis

Accomplishments:

- Demonstrated that immunization with a purine auxotrophic mutant of *Brucella melitensis* protects mice against subsequent airway challenge with virulent brucellae.
- Established an oral immunization regimen in mice using the purine auxotrophic mutant of *B. melitensis.*
- Demonstrated that two new genetically defined mutants of *B. melitensis* are attenuated for growth in macrophages and mice and evoke an immune response in mice.
- Demonstrated that intranasal immunization with a complex of *Neisseria meningitidis* Group B outer membrane protein (GBOMP) and *Brucella* lipopolysaccharide (LPS) protects mice against subsequent airway challenge with virulent brucellae.
- Demonstrated that two new preparations of GBOMP-LPS complex are immunogenic via the intranasal route in mice.
- Developed a new whole cell protein preparation from rough brucellae and showed that it elicits TH1 cytokine production from cells from animals immunized with all three new mutants of *B. melitensis*.
- Raised polyclonal antibody against whole cell protein prepared from rough brucellae to detect brucellae in tissues and for use in ELISA.
- Developed improved methods to quantitate cytokine mRNA and protein in cells responding to *Brucella* infection.
- Developed improved immunohistochemical and DNA hybridization methods to detect brucellae in tissues.
- Established a protocol to challenge nonhuman primates with *B. melitensis*.

THREAT CATEGORY: TOXINS

The countermeasures, technical barriers, and accomplishments in the biological threat category of protein toxins are outlined below.

Countermeasures:

- Antibodies (antitoxins) directed against common antigens of protein toxin molecules
- Vaccines for immunity against protein toxin threat agents
- Confirmatory assays to identify protein toxins specifically or classes of protein toxins
- Drugs for supportive therapy of agent intoxication
- Pharmaceuticals to delay or antagonize toxin effects.

*Technical Barriers**:***

- Capability to produce GMP pilot lots of vaccine candidates.
- Inability to perform human clinical trials to prove efficacy of vaccines and antitoxins.
- Difficulty in optimizing and comparing different expression vectors for recombinant products.

- Immunogenicity of vaccine and vaccine delivery technology.
- Difficulty in field testing diagnostic kits under natural conditions.
- Difficulty in producing polyvalent vaccines effective against classes of toxins.
- Lack of rapid confirmatory assays with "gold standard" sensitivity and specificity.
- Appropriate animal model systems for investigation of some protein toxin threats and countermeasures.
- Defining surrogate markers of protection.
- Appropriate model system for testing treatment efficacy and safety in humans.
- Lack of highly refined x-ray crystallographic structures of several protein toxins.

Botulinum Toxin

Accomplishments:

- Successfully produced and packaged in vials the first recombinant botulinum vaccine candidate, the Hc fragment from serotype B. Completed the genetic characterization of the Master Cell Bank and the Master Production Cell Bank, to include restriction enzyme mapping, copy number determination, DNA sequence analysis, etc.
- Produced monoclonal antibodies to botulinum neurotoxin serotype A that successfully protected experimental animals from the lethal effects of low levels of the toxin.
- Successfully identified a synthetic peptide based on a portion of serotype A toxin that protected mice against exposure to low levels of the toxin.
- Cloned the synthetic genes of the Hc region of botulinum neurotoxins serotypes C1, D, E, F and G into the yeast, *Pichia pastoris*, and initiated studies of the expression of these gene products and their ability to protect mice from lethal challenge with homologous serotypes of toxin.
- Cloned and expressed the light chains of botulinum neurotoxins A and B, purified the gene products and demonstrated that they retained their enzymatic activity, all as a prelude to ultimate determination of the structure of this portion of the botulinum neurotoxins.
- Studied the cellular immune response to botulinum toxoids by measuring transformation of peripheral lymphocytes from immunized and naive donors, and determined that a pattern of lymphocyte transformation correlated to the humoral immune response pattern.
- Demonstrated that the cleavage products of botulinum neurotoxin (BoNT) serotypes A and E have direct inhibitory actions on acetylcholine release when microinjected into *Aplysia californica* buccal ganglia.
- Determined that prolonged paralysis time following BoNT/A intoxication in mammalian skeletal muscle is due to long residence time of serotype A in the nerve terminal.
- Developed a novel biological membrane model for studying the ion channels formed by the heavy chains of BoNT/A and /E to test potential inhibitors of toxin internalization.
- Characterized the cytotoxic actions of zinc chelators on primary cortical neurons and clonal NG108-15 neuroblastoma-glioma hybrid cells. The membrane permeable chelator TPEN produced apoptosis followed by necrosis above, 5 μM, but had no toxicity at

lower concentrations.

- Determined that SNARE motifs in SNAP-25 and synaptobrevin (10-12 amino acids containing 3-4 acidic residues) served as low-affinity binding sites for zinc. Molecular modeling revealed that SNARE motif peptides resemble metal binding sites of magnesium- and zinc-binding proteins.
- Developed probes for Northern and Western blot analysis of synaptobrevin, SNAP-25 and syntaxin to determine the time course for induction of new mRNA and protein after destruction by BoNT/B and /A.
- Demonstrated the utility of the phrenic nerve-hemidiaphragm preparation for studying epitopes on the binding domain of BoNT heavy chain. Three principal epitopes were identified by single chain F_v antibody fragments.
- Developed a novel method for stabilizing the isolated light chain of BoNT/B by biotinylating free cysteine SH groups and coupling the complex to soluble avidin. This modified light chain exhibited approximately 10-fold greater stability at 4°C.
- Developed a rapid fluorescent microplate assay for monitoring the catalytic activity of BoNT/E using a 42 amino acid peptide distributed symmetrically around the BoNT/E cleavage site with biotin on the N-terminus and a fluorescent tag on the C-terminus.
- Tested 15 new metalloprotease inhibitors obtained via Material Transfer Agreements (MTAs) and Small Business Initiative Research (SBIR) for inhibition of BoNT/B. Two compounds were promising, with inhibition constants of approximately 50 µM.
- Determined that phosphorylation of BoNT/A by protein kinase C increased stability and metalloprotease activity suggesting- that inhibitors of phosphorylation may be of therapeutic benefit (Dr. M Montal. UCSD).
- Determined preliminary structure-activity profile for captopril-based active site inhibitors of BoNT/B metalloprotease activity (Promag Corp., SBIR Phase I).
- Determined preliminary structure-activity profile for phosphoramidon-based active site inhibitors of BoNT/A metalloprotease activity (Hawaii Biotechnology, SBIR Phase I).

Staphylococcal Enterotoxin

Accomplishments:

- Identified interleukin-8 (IL-8) as the first cytokine to appear in human macrophages stimulated with Staphylococcal Enterotoxin A (SEA) and Staphylococcal Enterotoxin B (SEB) *in vitro*.
- Developed a novel computational method for the rapid prediction and assessment of protein-protein associations and applied this approach to characterization of binding of recombinant SEA (rSEA) and recombinant SEB (rSEB) proteins with target T-cell receptors.
- Initiated studies to evaluate vaccine candidates, both toxoid and recombinant proteins, for effectiveness in both lethal and incapacitation animal models.
- Initiated development of additional animal models for measuring SE incapacitation, and identified increased levels of IL-6 and IL-2 in sera of monkeys exposed to a non-lethal dose of SEB.

- Initiated production under GMP conditions of a pilot lot of recombinant SEB vaccine candidate, and standardized a potency assay for this material.
- Initiated a study of the duration of immunity of rSEB vaccine in nonhuman primates.
- Completed a study of the immunogenicity of a combination SEB plus SEA vaccine in nonhuman primates.

Ricin

Accomplishments:

- Used computational chemistry and molecular modeling to examine the structure of ricin bound to its naturally occurring molecular target to derive information on the structural regions within the ricin molecule that could serve as targets for inhibitory drugs.
- Characterized the molecular structure of the neutralizing epitope of ricin - that portion which binds to protective antibody - in support of efforts to design potential peptide vaccines for ricin and other related toxins.
- Determined the stability and other characteristics of the candidate deglycosylated ricin A chain vaccine, and found that it is stable and potent when stored at a range of temperatures for at least a year, and that it appears to be safe as well as effective in animal models.

Clostridium Perfringens

Accomplishments:

- Characterized the toxicity of *C. perfringens* toxin types A, B, C, D and E when administered to mice and rats by either the parenteral or aerosol routes and found that toxicity was highly dependent on the dose and route of administration as well as on other technical parameters of the exposure.

THREAT CATEGORY: VIRAL AGENTS

The countermeasures, technical barriers, and accomplishments in the biological threat category of viral agents are outlined below.

Countermeasures:

- Vaccines for immunity against viral threat agents
- Antibodies and antivirals for treatment of viral disease
- Devices and technologies for diagnosis of viral disease

Technical Barriers:

- Appropriate animal model systems for investigation of viral threats and countermeasures
- Capability to produce GMP pilot lots of vaccine candidates

- Inability to perform human clinical trials to prove efficacy of vaccines
- Production of multivalent vaccines against heterologous viral agents
- Difficulty in optimizing and comparing different expression vectors for recombinant products (vaccines and antibodies)
- Immune enhancement of disease
- Rapid virus identification technology
- Defining surrogate markers of protection

Encephalitis Viruses

Accomplishments:

- Evaluated oral and nasal routes of immunization in mice with live-attenuated TC-83 Venuzuelan Equine Encephalitis (VEE) vaccine and the inactivated C-84 vaccine, and found that oral immunization did not induce immunity in the majority of mice, but that nasal delivery of the vaccine protected one strain of mice from aerosol or parenteral challenge with wild-type, virulent virus.
- Demonstrated conclusively that the VEE receptor protein in an insect cell line is the laminin receptor; cloned and expressed this receptor and demonstrated that antibody to the insect cell receptor does not cross-react with the receptor from vertebrate cells.
- Cloned, sequenced and expressed the complete structural protein regions of VEE 1A, 1E, Eastern Equine Encephalitis (EEE) and Western Equine Encephalitis (WEE) viruses; these regions include all known protective epitopes of these viruses and serve as the basis for evaluation of a multivalent encephalitis virus vaccine.
- Sequenced and analyzed 21 VEE 1E strains and 7 VEE III strains to determine the extent of genetic diversity, and found that a single vaccine for each type should be sufficient for protection against that serotype regardless of the geographic origin of the virus strain.
- Generated live-attenuated vaccine candidates for WEE and EEE viruses by site-directed mutagenesis of cDNA infectious clones.
- Tested genetically engineered WEE virus vaccine candidates in mice and found that they were avirulent, and induced protection against virulent virus strains.
- Tested EEE vaccine candidates in mice and determined that they were attenuated and immunogenic.
- Initiated GMP production of the selected VEE infectious clone vaccine candidate (V3526), and developed nucleotide sequencing and PCR assays to assist in quality assurance and monitoring of the production process.
- Created an infectious clone VEE IE vaccine prototype modeled on the IA vaccine candidate V3526, and determined that it was attenuated, highly immunogenic, and elicited protection against virulent challenge in animal models.

Variola, the Causative Agent of Smallpox

Accomplishments:

- Cloned individual vaccinia genes, expressed them in an RNA replicon vector, immunized animals and determined that high levels of neutralizing antibodies or cytolytic antibodies could be predicted from knowledge of the gene used for the immunization.
- Screened 18 antiviral drugs, which were selected to separately target six different functions involved in poxvirus replication, against a panel of orthopoxviruses, including monkeypox and smallpox (performed at the Centers for Disease Control and Prevention, Atlanta, GA), and selected the five most active compounds for *in vivo* studies.
- Developed an intranasal cowpox mouse model for use in drug evaluation, and characterized the disease pathogenesis in detail in order to compare to monkeypox and known characteristics of smallpox in humans.
- Assessed the value of the antiviral drug cidofovir (licensed for other indications) in the cowpox mouse model, and determined that one dose of drug prophylaxis was protective when begun as early as 12 days prior to infection; treatment was effective even when initiated as late as day 5 postinfection.
- Determined in nonhuman primates that cidofovir protected completely from clinical and laboratory signs of disease when animals were challenged by aerosol with monkeypox.
- Developed rapid, deployable PCR assays using real time fluorescence monitoring for orthopox viruses.
- Developed a computer-enhanced method to distinguish orthopoxvirus species and strains by long PCR RFLP analysis.
- Developed 5'-nuclease fluorogenic PCR assays which are capable of detecting single nucleotide differences in the hemagglutinin gene of orthopoxviruses using the ABI Prism 7700 sequence detector and a novel microchip device developed by Lawrence Livermore National Laboratory.

Filoviruses

Accomplishments:

- Determined that both the Marburg virus glycoprotein and nucleoprotein antigen are promising vaccine candidates that merit further testing in animal models.
- Initiated studies of the cell-mediated immune response to Ebola virus in mice in order to characterize the mechanisms by which T-cells may participate in protection from disease.
- Demonstrated protective efficacy of individual proteins or combinations of Ebola virus proteins in guinea pigs.
- Demonstrated in the mouse model of Ebola virus disease efficacy of a promising antiviral drug therapy.

Multi-agent Vaccines

Accomplishments:

- Using the RNA replicon vaccine vector system derived from infectious cDNA clones of Venezuelan equine encephalitis virus, constructed replicon vaccines containing genes from numerous filo- and bunya- viruses as well as for botulinum toxin Hc. These

vaccine constructs elicited protective immunity in animals when challenged with the corresponding agent.

- Constructed DNA plasmids suitable for "genetic immunization" for three viral agents and demonstrated protection from challenge in plasmid-immunized animals.

CONFIRMATORY ASSAYS FOR BIOLOGICAL WARFARE THREAT AGENTS

The accomplishments in the confirmatory assays for biological warfare threat agents are outlined below. The objective of this effort is to develop the capability to confirm in biological samples the initial field diagnosis of a biological warfare threat agent.

Accomplishments:

- Developed antigen capture ELISAs for SEA, B, C; botulinum toxin A and B.
- Developed sensitive and specific immunochromatographic hand-held assay for *Brucella spp.* and *F. tularensis*.
- Developed rapid, deployable PCR assays using real time fluorescence monitoring for *B. anthracis, Y. pestis,* and botulinum neurotoxin A and B.
- Developed recombinant antibodies to botulinum toxin A and B and to hemagglutinin. These reagents are being incorporated into assays including the immunochromatographic hand-held assay.
- Developed competitive ELISA for aflatoxin and T2 mycotoxin.
- Developed antigen capture ELISAs for Venezuelan equine encephalitis (VEE) and variola virus.
- Patented a novel multiple magnetic apparatus for processing of assay samples.
- Developed and demonstrated a high throughput, multi-well magnetic plate washing device for rapid sample preparation.
- Demonstrated an effective isolation procedure for biological threat agents from crude clinical specimens and environmental water samples.
- Developed solid phase fluorogenic and electrochemiluminescent immunoassays to comprehensively analyze the immune response of antibodies to bacterial spores and detection of toxins.
- Demonstrated low sample carry-over by multi-well immunomagnetic-fluorogenic assay system.
- Demonstrated 1-hour total assay time per 96 samples from sample-in to result read-out.
- Demonstrated 10- to 100-fold enhancements in immunoassay sensitivity for detecting biological threat agents.
- Demonstrated sensitive detection for various bacteria and toxins (*Bacillus anthrax* spores, Botulinum-type A, Ricin-A chain and Staphylococcal enterotoxin B).
- Developed rapid hand-held assays capable of detecting PCR products.
- Transitioned PCR biodetection capabilities to the Federal Bureau of Investigation.
- In collaboration with Lawrence Livermore National Laboratories developed a rapid, briefcase-sized PCR system.

- In collaboration with Battelle developed (breadboard) automated reader for hand-held immunochromatographic assays.
- Provided direct laboratory support, in-house or in the field, for: FBI, Secret Service, CBQRF, CBIRF, UNSCOM, and SOLIC.
- Provided technical assistance for hand-held assay construction to NATO and provided antibody reagents to NATO, and Joint Program Office.

D.2.3 Advanced Development Accomplishments

D.2.3.1 Botulism Antitoxin, Heptavalent, Equine, Types A, B, C, D, E, F, & G

- Studied and still being characterized as a replacement for the current IND treatment (IND #3703, Botulinum Immune Globulin, F(ab')2, Heptavalent, Equine) in treating the illness of botulism.
- Completed manufacturing of the first GMP lot of this botulism antitoxin for treatment against all seven known botulinum toxins.
- In response to the FDA's request for adventitious agent testing, this product was assayed under GMP guidelines in three cell culture systems.
- Conducted quarterly Stability Testing of the GMP product.
- Conducted a Pre-Investigational New Drug (IND) submission meeting with the FDA for this new GMP product.
- Provided Botulinum Antitoxin Standards to Battelle Medical Research and Evaluation Facility to be used in the development of the Pentavalent Botulism vaccine.
- Submitted a protocol for a Phase I Safety and Pharmacokinetic clinical study of this Botulism Antitoxin for Institutional Review Board review.

D.2.3.2 Botulism Immune Globulin (Human), Pentavalent (IND #1332)

- Conducted storage stability testing on this IND product.
- The IND remains open to accommodate emergency treatment requirements for exposure or possible exposure to botulinum toxins type A, B, C, D, or E.

D.2.3.3 Botulinum Type F Toxoid Vaccine (IND #5077)

- Continued the Phase 2 Safety and Immunogenicity clinical study of Botulinum Type F Toxoid Vaccine. The purpose of this study is to identify a vaccination schedule for the vaccine that is safe and maximally immunogenic.
- The 12-month serology after the primary three-inoculation series of vaccinations has been drawn from the last cohort in the Phase 2 study.
- The one-year booster phase of the Phase 2 study is under way.

D.2.3.4 Diagnostic Kit for Biological Warfare Agents

The accomplishments in the diagnostic assays for biological warfare threat agents are outlined below. The objective of this effort is to develop a rapid screening system for use in a

field medical laboratory (Hospital Level) to initially identify biological warfare agents in clinical samples obtained from exposed personnel. The kit will provide rapid information to the medical care provider that can be later confirmed using confirmatory assays that are more sensitive and quantitative.

- Prepared a Draft Analysis of Alternatives to support the decision-making process for the Diagnostic Kit for biological Warfare Agents program.
- Performance criteria for selectivity and sensitivity cutoff were evaluated as the means of comparison between alternatives.
- Obtained FDA input on guidance for the clearance of several Premarket Notification 510(k)s for the detection of a series of biological warfare agents from clinical samples.

D.2.4 Joint Vaccine Acquisition Program Accomplishments

The development of vaccines under this program involves studies which demonstrate product safety and efficacy and which are required for product licensure by the FDA. The Joint Vaccine Acquisition Program is managed by the Joint Program Office for Biological Defense. During FY97, the following actions were accomplished:

- The Request for Proposal for the prime systems contract was evaluated by a formal Source Selection Evaluation Board. The basic contract consists of development and licensure of three biological defense vaccine products, with options to develop and license 15 others and production options for all 18 vaccines. Contract was awarded on 7 November 1997.
- Assisted the Michigan Biologic Products Institute in identifying and correcting FDA compliance issues to ensure an anthrax vaccine stockpile, product integrity and future manufacturing capability.
- Received FDA Advisory Committee acceptance of the DoD strategy to use a surrogate model in lieu of human efficacy studies to support licensure of botulinum pentavalent toxoid vaccine. Preclinical studies to identify an appropriate surrogate model are ongoing. Human safety and efficacy studies are scheduled pending approval for indemnification of the contractor conducting the clinical studies.
- Managing the effort to amend the anthrax vaccine license to reduce the doses required to fewer than six inoculations, and to include indication for use in protection against an aerosol exposure.

D.3 MEDICAL NUCLEAR (RADIOLOGICAL) DEFENSE RESEARCH PROGRAM

D.3.1 Fielded Products

Advances in medical R&D significantly impact the warfighting mission by sustaining unit effectiveness through conserving the fighting strength of our service members. The individual service member whose performance is decremented by disease symptoms is significantly more likely to become a traumatic casualty. In this era of small, but highly lethal forces, loss of only a few team members can dramatically diminish a unit's capability. Medical R&D products (materiel and non-materiel solutions) provide the foundation that ensures the fielding of a flexible, sustainable, modernized force across the spectrum of conflict and in the full breadth and depth of the battlefield. Overcoming medical threats and extending human performance has provided a significant increase in military effectiveness in the past and presents the potential for future enhancement on military operational effectiveness. Some of the fielded materiel and non-materiel solutions by medical radiological defense R&D are:

- *Advances in the Treatment of Radiologic Injuries*, a medical research symposium publication, Pergamon Press, Elsevier Science, Ltd.
- North Atlantic Treaty Organization (NATO) Handbook AMedP-6, *Medical Aspects of Nuclear, Biological, and Chemical (NBC) Defensive Operations*
- Medical Effects of Nuclear Weapons Course--Training for approximately 760 Medical Department personnel in FY96.
- Advanced treatment modalities for bone marrow injury, such as the cytokines, which were available for the Gulf War
- New generation antiemetics effective for prevention of early debilitating symptoms of moderate radiation injuries (now being inserted into NATO doctrine)

D.3.2 Nuclear Defense Research and Development Accomplishments

The nuclear (or radiological) defense research and development technical barriers and accomplishments during FY97 are grouped in the following threat categories:

- Prompt radiation from nuclear weapons,
- Protracted low level radiation from fallout and other sources
- Combined effects of radiation and other factors

"*Prompt radiation*" refers to the high level radiation released by a nuclear weapon detonation in the first 60 seconds after the explosion. Significant injury occurs within seconds of exposure. "*Protracted low level radiation*" refers to radiation from nuclear fallout, radiological dissemination devices, and other sources which contaminate an area with radioactive particles. The exposure time required to cause casualties in this environment is much longer than the instantaneous exposure of prompt radiation. The "*combined effects*" environment significantly

augments the casualty rate by amplifying the subclinical effects of traditional trauma, burns, wounds, and infection. Due to the likelihood of an enemy's simultaneous use of nuclear dissemination weapons and chemical/biological agents, combined injury effects now also must include the previously unresearched interactions of low level radiation and chemical-biological weapons.

THREAT CATEGORY: PROMPT RADIATION

The countermeasures, technical barriers, and accomplishments in the threat area of prompt radiation are outlined below.

Countermeasures:

- Advanced medical treatment strategies for radiation injuries
- Drugs designed to increase resistance of soldiers to radiation and protect the soldier against radiation injury without compromising performance
- Drugs designed to prevent the onset of radiation-induced performance decrements such as fatigue, nausea, vomiting
- Assessment of radiation injury by biological dosimetry techniques

Technical Barriers:

- Known drugs that provide some radiation protective effects have serious performance-degrading side effects at drug doses required for operational requirements
- Mechanisms of action of several known treatment and radioprotective drug strategies are not well understood
- Drug delivery system which allows extended bioavailability is not available for radioprotectants

Status:

- Research in collaboration with pharmaceutical companies using large and small animal models is ongoing
- Research using cellular systems and rodents has begun to investigate strategies to mitigate against late effects *(e.g.,* cancer) of radiation
- Research using cellular systems and rodents has begun to investigate strategies to mitigate infection in irradiated animals
- Combination of drugs administered at non-toxic levels which provides protection has been identified
- Biological dosimetry techniques based on cytogenetic techniques are being validated and developed for fielding
- Greater emphasis is being provided on molecular and cellular biology strategies to elucidate mechanisms of radiation damage and protection
- Developing effective preventive treatments (cytokine-based) for lethally irradiated individuals

Accomplishments:

- Demonstrated in animal models improved medical treatment strategy that relies on use of pre-exposure prophylactic medications.
- Established protocol for post-exposure cytokine-based treatments that enhance standard clinical support.

THREAT CATEGORY: PROTRACTED LOW LEVEL RADIATION

The countermeasures, technical barriers, and accomplishments in the threat area of protracted low level radiation from nuclear fallout, radiological explosive devices, *etc.*, are outlined below.

Countermeasures:

- Advanced medical treatment strategies for protracted radiation injuries from both external and internal sources of radioactivity
- Drugs designed to protect personnel from the early and late effects of ionizing radiation without compromising performance
- Improved techniques to detect and remove internal sources of radioactivity
- Improved drug delivery system to provide protection during the entire period of radiation exposure

Technical Barriers:

- Availability of suitable radiation sources to study the effects of chronic exposure at relevant dose levels
- Difficulty in manipulating cellular repair mechanisms
- Toxicity of chelating agents used to remove sources of radioactivity
- Brief periods in which traditional radioprotective drugs are active
- Toxicity of radioprotective drugs used over protracted periods of time
- Lack of sustained drug delivery system for radioprotectants
- Microbial resistance to antibiotics

Status:

- New facility to permit protracted radiation exposure experiments is being planned to model current and future threat scenarios
- New biological models for internal and external cellular and whole-body chronic exposure studies are being developed
- New programs have been instituted for the study of molecular biology approaches to study gene radiation damage and repair mechanisms

- Novel drug delivery systems (*e.g.*, transdermal patches) are being evaluated for efficacy in providing protection in chronic radiation environments
- Develop effective antimicrobial treatment regimens for post-exposure use in radiation and combined injuries.

Accomplishments:

- Developed preliminary treatment protocols utilizing immune system stimulators that effectively guard against radiation-associated infections.

THREAT CATEGORY: COMBINED EFFECTS

The countermeasures, technical barriers, and accomplishments in the threat area of combined effects of nuclear radiation and trauma, burns, and infection are outlined below.

Countermeasures:

- Radiotherapeutic agents designed to decrease morbidity and mortality from multi-organ system failure due to the combined effects of radiation, trauma, burns, and infection
- Radioprotective drugs designed to harden the soldier against the effects of radiation, trauma, burns, and infection
- Combined therapeutic agents designed to decrease morbidity and mortality from and to enhance innate immune responses
- Computer models for predicting casualties following combined exposure to low levels of ionizing radiation and BW/CW agent aerosols

Technical Barriers:

- Availability of reliable animal models to predict effects in humans
- Antimicrobial resistance to current antimicrobial therapeutic agents
- Different sensitivities of biological systems at all levels to neutrons and gamma rays
- Mechanism of action of cell-growth factors is not well understood
- Sensitivity of bone marrow progenitor cells to low doses of ionizing radiation

Status:

- Research in collaboration with pharmaceutical companies using small and large animal models continues
- Evaluations of radioprotective and radiotherapeutic agents ongoing in mixed-field irradiated animal models
- New antimicrobial products under evaluation for the treatment of gram-positive and gram-negative bacterial sepsis in irradiated rodents.
- New immunomodulators evaluated for enhancing innate immune responses against infections.

- Molecular biology techniques utilized to understand the effects of radiation, trauma, and combined effects
- Molecular biology techniques utilized to understand the beneficial effects of cell growth factors, immunomodulators, and antimicrobial agents

Accomplishments:

- Sublethal irradiation significantly decreased survival and increased loss of body weight of mice orally challenged with a bacterial agent.

D.3.3 Predevelopment Products

Technical developments in predevelopment products for medical radiological defense include the following:

- Medical Effects of Nuclear Weapons CD-ROM interactive training program for military health care personnel
- Pre-Transition Information Paper: *Radioprotection by a Combination of Iloprost/Misoprostol/3D-MPL/WR-3689*
- Automated biodosimetry capability based on lymphocyte dicentric analysis.